



## SUBSTITUTE SPECIFICATION

Method and device for local probe microscopic visualization of a three-dimensional object

### 5 BACKGROUND OF THE INVENTION

The present invention relates to a method and a device of microscopic visualization of a three-dimensional object.

Nearfield microscopy techniques (STM - Scanning Tunneling Microscopy ; AFM - Atomic Force Microscopy ; SNOM - Scanning  
10 Nearfield Optical Microscopy ) are used whereof the principle consists in scanning a peak at the surface of the sample, enabling to obtain pictures with a resolution higher than that of the conventional optical microscopy.

These techniques have developed rapidly over the last  
15 years, but are only applicable to the survey of surfaces.

### SUMMARY OF THE INVENTION

The aim of the present invention is the realization of three-dimensional pictures thereby enabling visualization of  
20 the inside of a sample with a definition also higher than that permitted by the conventional optical microscopy.

Such a three-dimensional visualization offering nanometric resolution may receive numerous applications.

Generally speaking, it enables tracking of local probes  
25 included in structures.

In certain cases, this visualization consists of the representation of a slice, limited in depth, of the sample. In other cases, the accumulation of the pieces of information

contained in several slices enables to obtain global three-dimensional visualizations, for example in perspective.

Different applications of this visualization of local probes are possible.

5       The probes may be animated with limited movements within a structure.

      The analysis of the positions of the probes, of their statistic distribution, enables one to acquire knowledge on the structure, for instance on walls limiting the movements of  
10 the probes.

      Thus, the method and device of visualization subject of the present invention enable one to realise detailed pictures of the interstitial volume.

      This method still enables exploration of the structure of  
15 physiological elements such as cells like neurones, to describe the contact between two solid grains and to follow their evolution, to follow the dynamic diffusion of elements in soft matter or to perform temperature measurements of complex structures such as power electronic components.

20       When the probes are fixed, the study of their positions and of the possible evolution of these positions enables one better to know the medium wherein they are fixed and the external possible parameters to which they are subjected.

      In particular, it may be applied to the visualization of  
25 a colloidal gel whereof it will be possible to acquire accurate knowledge of the behaviour, for instance when it is subjected to homogeneous deformation.

One may thus study the structure of flocculated silica suspension. Indeed, by flocculation then concentration, it is possible to realise very regular and little dense silica aggregates, composed of spheres of approximately 50 nm in  
5 diameter.

To that end, the invention relates to a method of microscopic visualization of a three-dimensional object wherein the sample is visualised through an interferometer.

According to the invention, local probes of nanometric  
10 dimensions are inserted in the sample.

The probes are quite numerous, they are generally from 100 to several thousands in the observed field.

It has been seen that these local probes or particles may be animated by a movement whereof the time-related analysis  
15 enables the realization of characteristic pictures of the object. This movement may be the Brownian movement or it may be generated by acting on the probes, for instance by magnetic or electric effect.

The probes are of nanometric dimensions, i.e. generally  
20 smaller than 200 nanometers. They should diffuse the light. Thus, metallic probes sending back a significant proportion of the light that they receive in the opposite direction give good results.

In different preferred embodiments each exhibiting their  
25 specific advantages and liable to be combined together:

- the local probes are balls,
- the local probes are metallic,

- the interferometer is a Michelson interferometer,
- the interferometer is a Linnik interferometer,
- the interferometer is a Mirau interferometer,
- the interferometer includes a wide spectrum source,

5 By 'wide spectrum source' is meant here a source having a coherence length of the order of a micrometer.

- the source delivers short light pulses,
- optical means form the picture of a thin slice of the object on a matrix detector via the interferometer.

10 The thickness of the slice visualised is of the order of the coherence length of the source.

The invention also relates to a device of microscopic visualization of a three-dimensional object including:

- an interferometer,
- 15 - a wide spectrum source,
- a matrix sensor,
- means to form the picture of a thin slice of the object on the sensor via the interferometer,
- a unit for processing the picture produced by the
- 20 matrix sensor.

According to the invention, the device includes means for inserting local probes in the sample.

The light source is advantageously a pulse source which enables to fix the possible movement of the probes.

## BRIEF DESCRIPTION OF THE DRAWINGS

A particular embodiment of the invention will be described in detail with reference to the appended drawings wherein:

5       - Figure 1 is a representation of the device of the invention;

      - Figure 2 is a representation of the distribution of the energy received enabling localisation of a depth probe;

      - Figures 3A, 3B and 3C are schematic representations  
10   enabling to specify lateral localisation of the probes.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

On Figure 1, the sample has been represented in perspective and designated under the reference 1a with respect  
15   to the co-ordinates  $x$ ,  $y$ ,  $z$  then a side view of the reference 1 with respect to the plane  $xz$ .

The interferometer 2 is a Michelson interferometer composed of a semi-transparent blade 3, of a reference mirror 4, of a light source 5 and of a two-dimensional sensor 6  
20   defining two arms : the measurement arm 7 and the reference arm 8.

According to the invention, the local probes 9 or balls are inserted in the sample. They are particles of nanometric dimensions whereof the average dimension is smaller than  
25   200 nm, preferably ranging between 20 and 200 nm.

These probes are numerous, generally several thousands and at least one hundred in the field observed.

The voxel being the volume unit of the resolved object, one obtains good results when the probes are sufficient in number to be distributed in the volume observed, but sufficiently low so that, generally, one probe at most is  
5 present in a voxel.

The probes are in a medium such as a liquid, a gas or a gel. This medium must be transparent to observation wavelengths.

These probes 9 are preferably metallic balls,  
10 advantageously of gold or of silver.

They are animated by a Brownian movement while remaining inside a volume 10.

The light source 5 is advantageously a wide pulse source. The coherence width or length of the source determines, among  
15 others, the depth resolution. A pulse source enables to fix the possible movement of the probes 9.

The device thus enables one to acquire, at a given instant, the position of each of the probes inside the sample.

Indeed, the picture received by the two-dimensional  
20 sensor 6, preferably a CCD camera (Charge Coupled Device) or CMOS, provides for each probe a picture whereof the positioning in the plane xy of the sensor 6 is represented on Figures 3A, 3B and 3C. To this day, sensors having 1000 x 1000 pixels are common.

25 Figure 3A represents the pictures of each of the probes with respect to the contour 10 of the sample, Figure 3B is an enlarged representation of one of these pictures whereof the

central position is obtained by processing and then positioned in the plane xy as represented on Figure 3C.

The definition obtained in the plane xy depends on the definition of the sensor 6 and on the digital processing  
5 carried by the processing unit 11 to obtain the central position of each of the probes.

The depth positioning is obtained by interferometric techniques and represented on Figure 2. The depth measurement field is determined by the coherence length of the light  
10 which is advantageously low.

This field depth is itself divisible by analysis of the phase, each of the probes 9 producing a picture of different colour according to its position inside the field. Besides, it is possible to vary the relative positions of the sample and  
15 of the reference mirror, thereby modifying the position of the field, in depth, inside the sample.

It is therefore thus possible to obtain at each instant the three-dimensional visualization of the probes inside the sample. The accumulation of these pieces of information  
20 varying due to the Brownian movement to which the probes are subjected, enables one to obtain via the processing unit, the three-dimensional contour 10 of the sample.

The field depth is conventionally of the order of 1 micron and one obtains, by analysis of the phase, space  
25 localisation of the probes with a resolution of the order of some ten nanometers in each direction. Similarly, the sampling of diffraction spots enables localisation of their centres,

which are characteristic of the positions of the probes with enhanced accuracy. The interferometric techniques involve enable visualization of probes of a few ten nanometers in diameter which exhibit the equivalent of a reflection  
5 coefficient of approximately  $10^{-5}$  for visible wavelengths.

Different types of interferometer may be used whereas the description made above implements a Michelson interferometer, it is also possible to use a Linnik interferometer or a Mirau interferometer.